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<b>(21) International Application Number:</b> PCT/US90/05938 <b>(22) International Filing Date:</b> 16 October 1990 (16.10.90)  <b>(30) Priority data:</b> 422,392 16 October 1989 (16.10.89) US  <b>(71) Applicant:</b> GENELABS INCORPORATED [US/US]; 505 Penobscot Drive, Redwood City, CA 94063 (US).  <b>(72) Inventors:</b> LOVETT, Michael ; 416 Durant Way, Mill Valley, CA 94941 (US). REYES, Gregory, R. ; 2112 St. Francis Drive, Palo Alto, CA 94303 (US). WEISSMAN, Sherman, M. ; 495 St. Ronan Street, New Haven, CT 06511 (US). JORGENSEN, Linda, M. ; 555 Addison Avenue, Palo Alto, CA 94301 (US).		<b>(74) Agent:</b> DEHLINGER, Peter, J.; 350 Cambridge Avenue, Suite 100, Palo Alto, CA 94306 (US).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>With international search report.</i> <i>With amended claims.</i>  <b>Date of publication of the amended claims:</b> 10 June 1993 (10.06.93)
<b>(54) Title:</b> NON-SPECIFIC DNA AMPLIFICATION  <b>(57) Abstract</b>  A method of non-specifically amplifying different-sequence fragments in a mixture of duplex DNA fragments is disclosed. The fragments are provided with end linkers, and the mixture is amplified by successive primer-initiated replication. Also disclosed is a method of cloning cDNA species which are homologous to a region of contiguous genomic DNA and are selected from a mixture of cDNA species.		

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## AMENDED CLAIMS

[received by the International Bureau on 22 April 1991 (22.04.91);  
original claims 2 and 3 cancelled;  
original claims 1 and 4-15 renumbered as claims 1 and 2-13  
wherein claims 1, 11 and 14 are amended;  
claim 16 amended and renumbered as claim 15 (5 pages)]

1. A method of isolating from a mixture of  
cDNA fragments two or more specific cDNA fragments which  
5 are homologous to a contiguous region of genomic DNA,  
comprising  
obtaining the contiguous region of genomic DNA,  
preparing the mixture of cDNA fragments to  
contain end-terminal priming sequences,  
10 denaturing the fragments of the mixture to  
produce single fragment strands with end-terminal priming  
sequences,  
isolating from said mixture of fragments in  
single-strand form, those single fragment strands which  
15 hybridize to the contiguous region of genomic DNA,  
hybridizing the single fragment strands which  
are homologous to the genomic region with a primer whose  
sequence is complementary to said end-terminal priming  
sequences on each fragment strand, to form strand/primer  
20 complexes,  
converting the strand/primer complexes to  
double-strand fragments in the presence of polymerase and  
deoxynucleotides,  
denaturing the double-strand fragments, and  
25 repeating said hybridizing, converting, and  
denaturing steps until a desired degree of cDNA  
amplification is achieved.

2. The method of claim 1, wherein the  
30 contiguous genomic DNA section is bound to a solid  
support.

3. The method of claim 1, wherein the  
contiguous genomic DNA section is contained in yeast  
35 artificial chromosomes.

4. The method of claim 1, wherein the contiguous genomic DNA section represents the bovine leukosis virus genome and the mixture of cDNA species is  
5 a cDNA library made from mRNA obtained from a cell line infected with a virus derived from cell line 10C9.

5. The method of claim 1, for use in identifying cDNAs which correspond to genes located in  
10 the same chromosomal region as a known gene, wherein said genomic fragments are obtained by

(a) preparative size fractionating of genomic DNA fragments containing the known gene,

(b) ligating linkers to the genomic DNA  
15 fragments, which are useful as primers for sequence-independent amplification, to the ends of the DNA fragments and digesting with a restriction enzyme to eliminate the presence of redundant linkers on the ends of the DNA fragments,

(c) mixing the DNA fragments with DNA  
20 polymerase, all four deoxyribonucleotides, and primers homologous to the linkers present on the ends of the DNA fragments, and

(d) reacting the mixture under conditions to  
25 produce sequence-independent amplification of the DNA fragments.

6. The method of claim 5, wherein the preparative size fractionating of genomic DNA fragments  
30 containing the known gene involves identification of the DNA fragments of interest by adding primers homologous to the known gene, DNA polymerase, and all four deoxyribonucleotides, to the gel matrix and treating the gel matrix under conditions which promote amplification  
35 of the region of the known gene defined by the primers.

7. The method of claim 1, wherein the known gene is a growth factor or growth factor receptor gene.

5           8. The method of claim 7, wherein the known gene is an interleukin.

          9. The method of claim 8, wherein the known gene is IL-5 and the mixture of cDNA species is a T-cell  
10   cDNA library.

          10. The method of claim 7, wherein the known gene is erythropoietin and the mixture of cDNA species is a renal cDNA library.

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          11. The method of claim 1, wherein said preparing of cDNA species to contain end-terminal priming sequences includes

          attaching a double-strand linker to the  
20   fragments of the cDNA mixture,

          denaturing the fragments to produce single fragment strands with linker-strand ends, where the linker-strand ends serve as end-terminal priming sequences,

25           hybridizing the single strands with a primer whose sequence is complementary to a linker-strand end on each fragment strand, to form strand/primer complexes,

          converting the strand/primer complexes to double-strand fragments in the presence of polymerase and  
30   deoxynucleotides, and

          repeating said denaturing, hybridizing, and converting steps until a desired degree of amplification is achieved.

12. The method of claim 1, wherein said preparing of cDNA species to contain end-terminal priming sequences includes the cloning of the cDNA species into a suitable vector and using the known 5' and 3' vector sequences, which flank the cDNA insert, as end-terminal priming sequences.

13. The method of claim 12, wherein said preparing further includes the amplification of the cDNA species by mixing the cDNA species containing said end-terminal priming sequences with DNA polymerase, all four deoxyribonucleotide triphosphates, and primers homologous to the cDNA end-terminal priming sequences, and reacting the mixture under conditions to produce sequence-independent amplification of the single-stranded cDNA species.

14. The method of claim 1, which further includes cloning the amplified specific cDNAs into a vector.

15. A method of cloning two or more cDNA species which are homologous to a contiguous region of genomic DNA and are selected from a mixture of cDNA species, said method comprising,

(a) preparing cDNA species to contain end-terminal priming sequences,

(b) isolating single-stranded cDNA species on the basis of their hybridization to a first set of selected genomic fragments,

(c) mixing the isolated single-stranded cDNA species with DNA polymerase, all four deoxyribonucleotide triphosphates, and primers homologous to the cDNA end-terminal priming sequences,

(d) reacting the mixture under conditions to produce sequence-independent amplification of the single-stranded cDNA species,

(e) isolating single-stranded cDNA species,  
5 from the mixture of step (d), on the basis of their hybridization to a second set of selected genomic fragments,

(f) mixing the isolated single-stranded cDNA species of step (e) with DNA polymerase, all four  
10 deoxyribonucleotide triphosphates, and primers homologous to the cDNA end-terminal priming sequences,

(g) reacting the mixture under conditions to produce sequence-independent amplification of the single-stranded cDNA species,

15 (h) cloning the amplified cDNA species into a vector.